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Metabolism of 1-C^{14} Octanoic and 1-C^{14} Palmitic Acid by Rat Intestinal Slices.* (30565)

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The classic studies of Bloom and Chaikoff (1,2) and Borgström(3) demonstrated that after intestinal absorption, fatty acid molecules with chain lengths of 10 or less carbon atoms were transported primarily in the portal vein whereas fatty acids with chain lengths of greater than 16 carbon atoms were transported predominantly in lymph. Recent studies(4-7) have indicated that differences in the behavior of fatty acids during absorption are probably due to variations in their intramucosal metabolism as well as to differences in physical properties. However, these studies have dealt primarily with the processes of mucosal hydrolysis of glycerides and mucosal esterification of fatty acids. Little attention has been directed at the problem of catabolism of fatty acids by intestinal mucosa.

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In the present investigation studies were undertaken to define more clearly the differences in the mucosal metabolism of a long chain fatty acid (palmitic) as compared to a medium chain fatty acid (octanoic). The data obtained indicate that octanoic acid is catabolized to CO_2 and water soluble products to a greater extent than palmitic acid by rat jejunal slices. Analysis of the lipid soluble products by thin layer and gas liquid chromatography revealed additional differences in the metabolism of these 2 fatty acids.

Materials and methods. 1-C^{14} octanoic and 1-C^{14} palmitic acid (New England Nuclear Corp.) were purified by thin layer chromatography. The fatty acid substrates were dissolved in a small amount of ether and homogenized in 10% crystalline bovine albumin. Non-radioactive lipid carrier was added before homogenization so that the final concentration of the lipid-albumin suspensions was 10 micromoles lipid per ml.

Aliquots of the various lipid-albumin suspensions and other water soluble products were added to 12 ml of a counting solution prepared as previously described(8). The solution prepared for counting substances in organic solvents contained 15 ml of toluene with 0.04% liquiflor (Pilot Chemical Co.).

Radioassay was performed in a Packard Tri-Carb liquid scintillation spectrometer. Quenching was corrected for by utilizing a channels ratio method as described by Bush(9).

Female albino rats (Charles River Breeding Laboratories) weighing 160-200 g were fasted overnight before use in these experiments. The animals were killed by a blow on the head and the small bowel rinsed *in situ* with 50 ml of ice cold 0.85% NaCl. The upper half of the intestine was everted over a glass rod and intestinal slices prepared as previously described(8). Six jejunal slices weighing between .35-.40 g were added to each incubation flask which contained the following: 0.5 ml of 5-fold concentrated low calcium Kreb's-Ringer bicarbonate buffer, pH 7.4, 0.5 ml of 0.15M $NaHCO_3$, 7.5 μmoles of glucose in 1.0 ml water, and 1.0 ml of a lipid-albumin suspension containing either 1-C^{14} octanoic or 1-C^{14} palmitic acid. The flasks were gassed for 30 minutes with 95% O_2, 5% CO_2 prior to addition of the jejunal slices and lipid substrates. In each experiment control flasks were used in which 0.25 ml of 10N H_2SO_4 was added at the start of incubation. The incubations were carried out in stoppered flasks at 37°C for 30 minutes and were terminated either by addition of 0.25 ml of 10N H_2SO_4 or placement in ice.

To measure the conversion of 1-C^{14} octanoic and 1-C^{14} palmitic acid to $C^{14}O$, 0.25 ml of 10N H_2SO_4 was added to stoppered flasks that were cooled in ice for 15 minutes after which 1.0 ml of hyamine 10-X was added to the center wall. The flasks remained in ice for 30 minutes after which the hyamine was removed and aliquots assayed for radioactivity.

When lipid soluble products were analyzed, the reactions were terminated by placing the flasks in ice. The tissue slices were removed, rinsed in distilled water, and blotted dry. The tissue slices and media were extracted separately by the method of Folch *et al*(10). In experiments with palmitic acid as radioactive substrate, the solvents containing the extracted lipids were evaporated to dryness under a nitrogen stream at room temperature and the residues redissolved in chloroform. In the experiments with octanoic acid, the chloroform-methanol added at the end of the incubation contained 42 μmoles of carrier octanoic acid and 6 μmoles of carrier trioctanoin and the pH during the Folch extraction was adjusted to below 3. Because of the volatility of octanoic acid, the solvents containing the lipid extracts were evaporated slowly under a nitrogen stream in an ice bath. The dried extracts were then resuspended in chloroform. Aliquots of the lipid soluble products were assayed for radioactivity and also analyzed by thin layer and gas liquid chromatography.

Gas liquid chromatography analyses were performed using a Barber-Coleman model 25C equipped with an Argon ionization detector. The gas stream was split so that 25% was directed to the mass detector and 75% to the radioactive counter (fraction collector). The inlet pressure was 30 p.s.i. and the flow rate 80 ml per minute. Radioassay was performed by the method of Karmen *et al* (11) utilizing a Packard liquid scintillation spectrometer. For each analysis 5 μg of material in 5 μL was injected into the column. The column was coiled, 6 ft by 1/8 inch in diameter. The liquid phase was either 15% apiezon L or 20% ethylene glycol succinate and the solid support 80-90 mesh anakron. The column temperature was 197°C, the flash heat temperature 285°C, and the cell temperature 200°C.

Results. The data shown in Table I and Fig. 1 indicate that small intestinal slices *in vitro* metabolize 1-C^{14}-octanoic acid different-

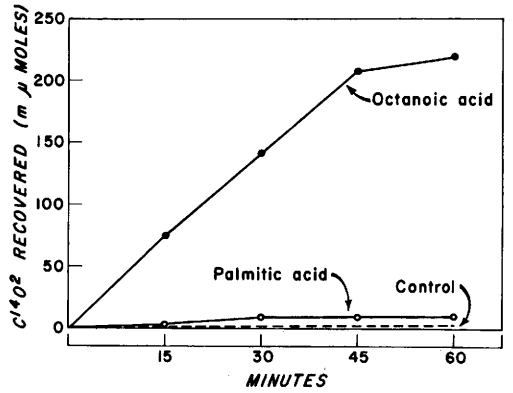

FIG. 1. Recovery of $C^{14}O_2$ after incubation of 1-C^{14} octanoic and 1-C^{14} palmitic acid with jejunal slices. Incubations were carried out for 15, 30, 45, and 60 min under conditions as described in *Methods.*

TABLE I. Metabolism of 1-C^{14} Octanoic and 1-C^{14} Palmitic Acid to $C^{14}O_2$, Water Soluble, and Lipid Soluble Products by Bowel Slices.

Lipid substrate (10 μmoles)	No. experiments	Distribution of recovered radioactivity (%) $C^{14}O_2$	Water soluble products	Lipid soluble products	Total recovery of administered radioactivity (%)
Octanoic acid	4	1.66 ± .12 *	2.09 ± .8	96.25 ± .8	84–98
Palmitic "	4	.097 ± .003	.106 ± .009	99.80 ± .02	92–98
p value		<.01	<.01	<.01	

The component of incubation mixtures and conditions of incubation were as described in *Methods*.

* Mean ± 1 S.D.

ly than 1-C^{14}-palmitic acid. A significantly greater amount of octanoic acid was oxidized to CO_2 and water soluble products and less was recovered as lipid soluble products compared to palmitic acid. The lipid soluble products from these studies were also analyzed by thin layer and gas liquid chromatography (Tables II and III) and additional differences in the metabolism of octanoic and palmitic acids by jejunal slices demonstrated. The data in Table II indicate that a significantly greater amount of the administered palmitic acid was esterified to triglyceride as compared to octanoic acid (33% *vs* 14%; p = <.01). Furthermore, there was also a greater incorporation of palmitic acid into total neutral lipid compared to octanoic acid (41% *vs* 19%; p = <.01).

The data in Table III indicate that after incubation of C^{14}-palmitic acid with jejunal slices 96% of the label was recovered in the form of palmitic acid as determined by gas liquid chromatography. In contrast, after incubation of C^{14}-octanoic acid with jejunal slices, a small but significant fraction of the label was recovered in fatty acids other than octanoic acid. As indicated in Table III, 3.7% was recovered as decanoic acid and 4.9% was incorporated into fatty acids with chain lengths of 12-20 carbon atoms.

Discussion. A considerable amount of evidence has accumulated indicating that medium chain and long chain fatty acids are metabolized differently by the intestinal mucosa, and this information has recently been summarized(12). It has been demonstrated that a medium chain fatty acid such as octanoic is poorly incorporated into mucosal triglycerides as compared to long chain fatty acids such as myristic, palmitic, and stearic(4-7). This has been shown to be due to several factors: (1) medium chain fatty acids are poorly activated to CoA thiolesters(13,14); (2) medium chain monoglycerides are rapidly hydrolyzed by a mucosal monoester hydrolase(15,16); and (3) medium chain triglycerides may also be hydrolyzed by a mucosal lipase system(7). As a result of the low activity of mucosal esterifying enzymes towards medium chain fatty acids and the presence of an active mucosal hydrolytic system directed towards medium chain monoglycerides and triglycerides, medium chain fatty acids accumulate in the intestinal mucosa predominantly in the form of non-esterified fatty acids regardless of whether medium chain fatty acids *per se* or medium chain triglycerides are administered. In contrast, long chain

TABLE II. Thin Layer Chromatography Analysis of Lipid Soluble Products After Incubation of 1-C^{14} Octanoic and 1-C^{14} Palmitic Acid with Jejunal Slices.

C^{14}-lipid substrate (10 μmoles)	No. experiments	Analysis of	Lipid recovered (μmoles)	Distribution of radioactivity (%) Monoglyceride & phospholipids	Diglyceride	Fatty acid	Triglyceride	Neutral lipid
Octanoic acid	4	Medium	7.836 ± .18*	.2 ± .17	.2 ± .1	99.4 ± .2	.2 ± .1	.6 ± .1
	4	Tissue	.806 ± .09	2.7 ±1.50	2.6 ±1.5	80.6 ± 8.5	14.0 ± 5.9	19.4 ± 8.5
Palmitic acid	5	Medium	8.970 ± .2	.4 ± .1	1.2 ± .2	94.6 ± 1.4	3.8 ± 1.3	5.4 ± 1.3
	5	Tissue	.905 ± .08	4.4 ± .3	3.8 ± .5	58.8 ± 7.2	33.0 ± 7.5	41.2 ± 7.5

* Mean ± 1 S.D.

TABLE III. Gas Liquid Chromatography Analysis of Lipid Soluble Products Obtained After Incubation of Jejunal Slices with Either 1-C^{14} Octanoic Acid or 1-C^{14} Palmitic Acid.

Lipid substrate	Analysis of	No. experiments	Distribution of radioactive label in fatty acids (%) 8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	20:0	Recovered radioactivity, dpm × 10^{-3}
1. Octanoic acid	Medium	2	99.0										9.2
	Tissue lipids	3	91.1	3.7	———	———	———	4.9	———	———	———	———	2.8
2. Palmitic acid	Medium	2					99.0		2.0	<1.0	<1.0	<1.0	8.0
	Tissue lipids	4	———	———	<1.0	———	95.6	<1.0					

fatty acids are present in intestinal mucosa primarily in the form of triglycerides after administration of either long chain fatty acids or long chain triglycerides(4-7).

The data obtained in the present study indicate that a significantly greater amount of palmitic acid was esterified to triglyceride and neutral lipids by intestinal mucosa as compared to octanoic acid. However, it is noteworthy that after incubation of 10 μmoles of octanoic acid with jejunal slices, 0.156 μmoles were recovered as neutral lipid (Table II). This value is somewhat higher than has been reported by others(4,5) and could possibly be related to the following factors: (1) tissue slices were used which may be more active than tissue homogenates in the esterification of fatty acid substrate(4); (2) larger amounts of lipid substrate (10 μmoles) were utilized in comparison with previous studies; and (3) cold carrier lipid was used to minimize loss of radioactivity during the extraction process.

It was of interest that octanoic acid was catabolized by intestinal mucosa to a greater extent than palmitic acid. This was also reflected by the observation that 9% of the C^{14} label from octanoic acid was incorporated into fatty acids with chain lengths of 10-20 carbon atoms (Table III). This suggests that some of the octanoic acid may first have been catabolized to 2 carbon fragments (*i.e.*, acetate) which were subsequently incorporated into long chain fatty acids. Since medium chain fatty acids are poorly activated to CoA thiolesters(13,14), it seems unlikely that the octanoic acid underwent elongation reactions to yield the longer chain fatty acids.

Summary. Measurements were carried out on the *in vitro* metabolism of C^{14}-octanoic and C^{14}-palmitic acid by rat intestinal slices. Significantly more octanoic acid was catabolized to CO_2 and water soluble products and less recovered as lipid soluble products compared to when palmitic acid was the substrate. Analysis of these lipid soluble products by thin layer chromatography revealed that more of the palmitic acid was esterified to triglyceride compared to octanoic acid. Further analysis of the lipid soluble products by gas liquid chromatography demonstrated that a small but significant amount (9%) of the label from C^{14}-octanoic acid was recovered in fatty acids with chain lengths of 10-20 carbon atoms. This suggests that some of the C^{14}-octanoic acid may have been catabolized to C^{14}-acetate which was then incorporated into long chain fatty acids. In contrast, virtually all of the label from C^{14}-palmitic acid was recovered as such, indicating that very little mucosal catabolism of palmitic acid had occurred.

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The Presence of Lymphocytes in Long Term Cultures of Newborn Mouse Thymic Epithelium.* (30566)

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Observations of *in vitro* cultures of newborn thymus fragments followed for as long as 120 days have been reported(1). We will report here the observations on cultures derived from newborn mouse thymus fragments initially explanted in a plasma clot and followed for over 22 months. The term epithelial cell, instead of that of reticulum cell employed in our previous work, will be used in the present note to describe the cells originating from the epithelial reticulum of the thymus. This appears justified both by the morphological appearance of the cells observed in culture and by the embryological derivation of the thymus(2,3,4,5,6,7).

Material and methods. Source of the thymus was a 24-hour-old MA mouse. The mouse was killed with ether and the thymus aseptically removed to a Petri dish containing Hanks' BSS. After washing, the thymus was transferred to a second Petri dish containing Hanks' BSS and finely minced. The minute fragments were then transferred to 2 Leighton tubes (Bellco Glass Co., Vineland, N. J.) into which a coverslip had been previously inserted. Two drops of chicken plasma (Difco) were pipetted to the coverslip before inserting the thymus fragments into the tube. The plasma solidified rapidly after transfer of the fragments and the medium was then added. This procedure favored the adhesion of the fragments to the glass.

The culture medium was Hanks' BSS with phenol red as indicator, plus vitamins, glutamin, essential amino acids and was supplemented with 11% fetal bovine serum (Microbiological Associates, Bethesda, Md.). No antibiotics were used. The medium was first changed after 4 days and thereafter changed every 5-7 days. Every 2 changes, after the old medium was discarded, the culture was washed with Hanks' BSS before the fresh medium was added.

Of the original cultures, one was sacrificed

FIG. 1. 4-day-old culture. Macrophages phagocytizing lymphocytes and debris, surrounded by dead or degenerating lymphocytes. 250 ×.
FIG. 2. 14-day-old culture, large lymphocytes growing on background of a continuous sheet of epithelial cells. Original magnification 250 ×, enlarged 3 times. Phase contrast.
FIG. 3. 14-day-old culture. Lymphocytes growing on epithelial cells. May-Grünwald and Giemsa, 250 ×.
FIG. 4. 15-month-old culture. Mitosis of an epithelial cell. Original magnification 250 ×, enlarged 3 times. Phase contrast.
FIG. 5. 22-hour-old subculture from the 14-month-old original culture. Colony of epithelial cells. May-Grünwald and Giemsa, 110 ×.
FIG. 6. 18-hour-old subculture from the 14-month-old original culture. Small colony of epithelial cells. A few lymphocytes with pyknotic nuclei are visible. One is engulfed in a cytoplasmatic vacuole. May-Grünwald and Giemsa, 250 ×.

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